UNIVERSITÀ DI PISA

PhD course in Molecular Biotechnologies

XX cycle (2005-2007)

PhD Thesis

"Hyaluronan role in *Xenopus laevis* visceral skeleton morphogenesis"

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PUBLISHED PAPERS:

1. Ori M, Nardini M, Casini P, Perris R, Nardi I. (2006). XHas2 activity is required during somitogenesis and precursor cell migration in Xenopus development. *Development*. **133**, 631-40.

2. Casini P, Ori M, Avenoso A, D'Ascola A, Traina P, Mattina W, Perris R, Campo GM, Calabroni A, Nardi I, Campo S. (2008, submitted to *Int. J. Dev. Biol*.). Identification and gene expression of versican during early development of *Xenopus.*

PREFACE

During the first year of my PhD programme I took part in a project dealing with the identification of *XCD44* gene expression pattern and its functional role during *Xenopus laevis* muscle development. These results are reported in the last part of this thesis as publications in *"Development"* journal (Ori, Nardini, Casini *et al.,* 2006).

This PhD thesis describes all the work I have done in the last two years. In this period my efforts were focused on the project "Hyaluronan role in *Xenopus laevis* visceral skeleton morphogenesis" that is the subject of this manuscript. Moreover, as complementary studies, I report in the thesis the expression patter of the *Xversican* and *XRHAMM* genes during *Xenopus laevis* development. The *Xversican* cDNA has been cloned in collaboration with the group of Prof. Calatroni, University of Messina and the data regarding the identification and the gene expression of this gene are reported in the manuscript attached at the end of this thesis (Casini *et al.*, 2008) actually submitted to an international peer review journal ("*International Journal Developmental Biology"*).

ABSTRACT

Hyaluronan is a crucial glycosaminoglycan of vertebrate extracellular matrix. In dynamic cellular systems, such as embryonic development, tissue regeneration and tumorigenesis, hyaluronan has been shown to influence cell behaviour, including cell migration, proliferation and differentiation both by assembling the interstitial matrices and by directly influencing cell behaviour via interaction with signal transducing receptors such as CD44.

We are using *Xenopus laevis* to study the role of hyaluronan and CD44 *in vivo* during cell migration and differentiation processes. The spatio-temporal gene expression profile of the three known vertebrate hyaluronan synthases (*Has1*, *Has2* and *Has3*) shows a very close conservation of *Xenopus laevis Has* genes with that of mammals. Recently, we demonstrated a critical role of *XHas2* and *XCD44* during muscle formation and precursor muscle cell migration. To further dissect the role of these molecules on migration and differentiation processes, during my PhD programme I then focused my attention on cranial neural crest cells (NCCs) development, knocking-down the *XHas1, XHas2* and *XCD44* gene functions. I showed that the hyaluronan synthases and the hyaluronan receptor present a dynamic expression pattern during cranial NCCs development suggesting multiple roles in the various steps of cranial NCCs migration and differentiation. I demonstrated that XHas1 and XHas2, in concert with XCD44, are involved in the NCCs migration and that hyaluronan, but not XCD44, is required in post-migratory stages to support cells survival. In order to investigate possible action mechanisms underlying hyaluronan function, I started to explore the possible functional interactions of hyaluronan with alternative receptors, such as RHAMM, and hyaluronan binding proteins such as the protoglycan versican.

On the whole the presented data demonstrated an unsuspected critical role of hyaluronan in the visceral skeleton morphogenesis and in particular in NCCs migration and differentiation. Moreover I showed for the first time the gene expression pattern of *Xversican* and *XRHAMM* in *Xenopus laevis* opening new working hypothesis that will be further investigated in the near future.